## REMARKS

### Claim Amendments

Claims 1, 2, 4, 6, 8, 9 and 14-16 are present in the application. Claims 3, 5, 7, and 10-13 are canceled. Claims 6, 8, 9 and 14-16 are now withdrawn. Claims 1, 2, and 4 are subject to examination. Claim 1 is amended.

Claim 1 is amended to address the objection raised to Claims 1-2 and 4.

### Claims rejection under 35 USC §103(a)

Claims 1, 2, and 4 are rejected as obvious over Takashima et al. (US2002/0051926 or the "'926 application") in view of Caputo et al. (US2002/065421 or the "'421 application"), Stavrianopoulos et al. (US2003/0225247 or the "'247 application"), and Greg T. Hermanson, *Bioconjugate Techniques,* (1996), P.228-229, and 287 (the "Hermanson") and the incorporated references.

Applicant respectfully traverses.

The compounds of present (and past) Claim 1 have two essential features: (a) they comprise always **two different** functionalized linkers (i.e. having a terminal functional group capable of reacting) on the two indolenine ammonium atoms: $R_1$ and $R_2$ and (b) one of these linker if always functionalized with an alkyne group: -C≡CH.

It appears that the Examiner considers now **Takashima (US'926)** as the closest prior art. Applicant first corrects a statement made in the rejection concerning the teaching of US'926. Contrary to the statement on page 5 of the action, US'926 does not teach that "$R^{13}$ is a substituted alkyl, wherein the substituents are carboxyl group, a cyano group, a hydroxyl group, amino group, a heterocyclic group, sulfo group, an aryl group". This allegation is at best a very specific selection made from a much broader generalized teaching of US'926, which the reference itself does not provide.

As previously established by Applicant, US'926 discloses indocyanine molecules having either only one functionalized linker or two identical functionalized linkers. US'926 makes no teaching or suggestion of, and offers no other teaching as motivation for, two different functionalized linkers. The possibility of having two different linkers is a mere theory, which is

weakened not only by the fact that all 35 compounds punctually described on pages 7, 8, 9 and 10 have two identical linkers in positions $R^1$ and $R^2$ of Formula (1) [i.e. -C≡CH-$R^{18}$ and $R^{13}$ of Formula (3)], but also by the explicit affirmation in paragraph [0065]: "*The aliphatic group and aromatic group have the same meaning as the aliphatic group and the aromatic group respectively represented by $R^{18}$. Particularly preferable is case where $R^2$ represents the same group as $R^1$*". As a matter of fact, all the reported real compounds have identical $R^1$ and $R^2$, both comprising a -C≡C- group.

This is consistent with, if not required by, the objectives for the US'926 compound as a "polymerizable compound having an ethylenically unsaturated bond" (emphasis added), for use as a photo-polymerizable dye in a polymerizable composition containing a radical generator. This dye should have high sensitivity not only to UV light but also to visible-to-infrared light, and should be highly decomposable by a radical, and excellent in decolorization (see paragraphs [0012] through [0016]). US'926 specifies that the photo-polymerization dye functions to spectrally sensitize the radical generator when irradiated by visible-to-infrared light. The polymerizable dye, in turn, is highly decolorized by the irradiation, so as not to interfere with the image on a recording material that utilizes the photopolymerizable composition.

US'926 uses three columns of the patent (paragraphs [0048] to [0064]) to describe the possible alkyl, alkenyl, alkynyl, aralkyl, and substituted alkyl, alkenyl, alkynyl, aralkyl, for the –$R^1$ moiety, which represent an exceedingly large number (perhaps, an unlimited number) of moieties for $R^{13}$ and $R^{18}$. Accordingly, in order to arrive at an indocyanine dye as provided in claim 1, starting from the teaching of US'926, one should firstly select among all the unlimited possible meanings of $R^{18}$ the meaning "H" in order to have a linker terminating with the group -C≡CH in $R^1$; secondly one should select among the unlimited number of possible meanings of $R^{13}$ a substituted alkyl group, having a reactive functional terminal group as in -$R^8$-Y of the present claim 1.

This artificial activity of selection and combination would be possible only with the hindsight provided by the Applicant's present invention, and would otherwise amount to an attributed teaching that the US'926 reference neither makes nor suggests, and which in fact flows contrary to the teaching in US'926, which does not describe or suggest a single compound having

$R^1$ different from $R^2$ (i.e. $R^{18}$ different from $R^{13}$) or a single compound having as $R^{13}$ a reactive group comparable to the meaning of "Y" in present claim 1.

For this reason the skilled person would have found no motivation to modify the compound of US '926 in the direction of Applicant's invention.

Finally, a conclusion of obviousness requires a rationale supported by predictability, that the combined or modified elements or techniques yield predictable results. The rejection fails to show that one of ordinary skill in the art would find it predicable that a compound of US'926, modified as suggested by the examiner to include a second different linker that does not have the required alkene or alkyne linker of the first linker, would retain all of the properties and features required of the polymerizable dye as discussed above. Applicant asserts that a more reasonable view is that a person of ordinary skill in the art would expect that the specific features of the dyes according to US'926 could not predictably be maintained by replacing the alkene or alkyne linker, which provides polymerization, with a hetero second linker.

Nor would the skilled practitioner have found any motivation in **US'421 (Caputo)** to modify the indocyanine dyes of US'926 to produce the indocyanine dyes of the present invention having two different linkers with <u>different reactivity.</u>

In fact, US '421 claims and describes asymmetrical indocyanines having always one single functionalized linker, therefore capable of bonding only one molecule (either a biomolecule or dye). Considering formula (I) of claim 10, or any of the compounds of claim 11, or any of the compounds of claim 14, or even any of the compounds illustrated in figures 4 to 11, one understands immediately that when one of the substituents on either of the two indolenine ammonium atoms is functionalized, the other is not functionalized: namely it is a simple ethyl group ($-CH_2-CH_3$), which, as is well known, is inert.

Moreover, when one functionalized linker is present on the benzyl ring of one of the indolenine moieties, then neither of the two indolenine ammonium atoms, corresponding to present $R_1$ and $R_2$ positions, are functionalized, both bearing an inert ethyl group.

Moreover, none of the US '421 compounds comprise one linker, in a position corresponding to either $R_1$ or $R_2$, that is functionalized with a $-C\equiv CH$ group.

For this reason, the skilled practitioner aware of the teaching in US '421 would not have found motivation to modify, or predictability in modifying, the dye of US '926 in order to produce a dye with two linkers having different reactivity.

As to **US'247 (Stavrianopoulos),** this document discloses a detection test for biomolecules (DNA, RNA, etc) in which the different hybridisation partners are labelled with a dye.

The Examiner proposes the combination of US '926 with Stavrianopoulos (US'247) affirming that this latter would suggest the use of cyanine dyes for labelling biomolecules (RNA, DNA, proteins, peptides). Applicant does not contest this finding, which however is completely irrelevant in assessing the inventive merit of the present invention.

Actually Applicant does not claim to have discovered that a biomolecule can be labelled with a cyanine dye, which possibility has been known for a long time. Applicant does claim to have produced new bifunctional cyanine dyes having two different reactive linkers exhibiting different reactivity.

This type of marker enables one to selectively link the cyanine dye to a first biomolecule and then to link the complex to a second biomolecule.

Actually, US '247 addresses a specific problem, that the labelling with a marker of nucleotides or biomolecules may be detrimental to the functionality of the labelled substance. See paragraphs [0022] and [0026], and specifically paragraph [0027] where it is affirmed:

> "[0027] *Attempts to limit this deleterious interaction has been carried out in several ways. For instance, attachment of the arm to the base has been carried out with either double bond alkene group ... or a triple bond alkyne group...*".

The solution proposed by US '247 is the use of reactive linking groups (R) capable of forming a **carbon-carbon linkage** with the target molecule, as detailed in paragraphs [0055] through [0058].

As specified in paragraph [0123], this new linkage offers advantages (i.e. less detriment to the target's functionality and higher efficiency) over those of the prior art. In particular it is affirmed in paragraph [0123]:

*"One aspect of the present invention discloses novel labeling agents .... capable of creating a carbon-carbon bond between a marker or label and a desired target molecule.* ***This is in contrast to labeling reagents described in the prior art which employed .... formation of a bond between an amine, sulphydryl, or hydroxyl group and an appropriate reactive group.*** " (emphasis added)

Examples of reactive groups forming a carbon-carbon bond are alkenes, alkynes and other (see paragraph [0125] of US'247).

Therefore, whenever a cyanine dye is selected as the labeling marker (see [0135]), either as mono- or bi-functional marker (see [0137]), this is always functionalized by way of a reactive groups capable of forming carbon-carbon bonds, such as -C≡C-, C=C- or other.

Actually the teaching of US'247 is identical to the teaching of US '926 that already discloses bifunctional cyanine dyes, all having two linkers with a terminal -C≡C- reactive groups.

Yet, it is important to stress here that one of the two linkers of the cyanine dyes of the present invention, that represented by the group $R_8$-Y (or $R_3$ to $R_6$) creates with the target molecules bonds which are -C-O- or -C-N- or -C-S- bonds, thus linkages of that type that the teaching of US'247 tries to avoid.

For this reason, the skilled practitioner aware of the teaching in US '926 would not find in US '247 any teaching, suggestion or motivation to modify, nor an predictability in modifying, the cyanine dyes of US '926, by replacing one of the two identical linkers with a different linkers represented by the group R2, including R8-Y, in the present invention.

Finally the Examiner cites the **Hermanson** reference. This document is a general disclosure of the concepts of hetero-bifunctional or -trifunctional cross-linkers used to cross-link proteins or other macromolecules.

First, the introduction into the present rejection of a new reference to show that the hetero bifunctional cross-linkers do indeed exist, would confirms that none of the previously discussed documents (US '926, US '421 and US'247) disclose a cyanine dye that is hetero-bifunctionalized.

Applicant admits that heterobifunctional cross linkers were known *per se*, and does not pretend to have invented the concept of "hetero bifunctional cross-linkers", but has invented new cyanine dyes highly suitable as labeling biomolecules.

Beyond disclosing the existence of heterobifunctional and trifunctional linkers, Hermanson neither teaches or suggest dyes (even less, cyanine dyes) nor addresses the problem of labeling with dyes material, such as nucleotides or polynucleotides, which must preserve its functionality, namely its capability to be used by a polymerase or to hybridize with a complementary polynucleotide.

Therefore, the skilled practitioner would not have found in Hermanson guidance or motivation to step beyond the express teaching of US '926, to transform the symmetrical bifunctional dyes disclosed in US'926, into the asymmetrical bifunctional cyanines of the present invention.

Lastly, the rejection refers to "and the incorporated references". For the record, the rejection makes no reference to any specific references that may have been incorporated into the references, and therefore asserts that rejection cannot in fact be based upon or supported by any such incorporated references.

In conclusion, none of the prior documents discussed by the examiner, taken alone or in combination would render the subject matter of claim 1 obvious.

**Claim objections**

The Examiner objected to Claim 1 as providing an incorrect Markush group. Applicant has amended claim 1 as suggested by the Examiner, rendering the objection moot.

The Examiner has also objected to Claim 1, 2, and 4 as containing both elected and non-elected subject matter.

**Rejoinder**

In view of the present amendments and arguments, the elected subject matter appears to be allowable over the prior art of record, and Applicant requests that the remaining non-elected subject matter of the claims, including withdrawn claims, be rejoined and examined if Claims 1, 2 and 4 are found allowable.

**CONCLUSION**

Applicant believes a full and complete response to the Office Action has been made. In view of the above remarks that traverse the rejections, allowance of all the claims is respectfully requested.

Respectfully submitted,

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